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PRINCIPAL INVESTIGATOR: Andrea M. Allan, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico Health Sciences Center
Albuquerque, NM 87131-0001

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14. ABSTRACT
We hypothesized that over-expression of 5-HT3 receptors decreases alcohol consumption because the presence of an increased number of 5-HT3 receptors increased the potentiation of dopamine release at lower alcohol concentrations. Thus, the animal requires less alcohol to obtain the same behavioral effect. Thus, the lower level of alcohol consumption seen in the 5-HT3 receptor over-expressing mice may be the result of increased inhibitory control over alcohol consumption We found that the 5-HT3 receptor over-expressing mice fail to behave aggressively in an intruder aggression test. They display less learned helpless behavior than wild type mice and have greater neuronal survival. We examined the impact of 5HT3 receptor over-expression on alcohol preference using a two-bottle free choice test and the impact of 5HT3 receptor over-expression on natural aggressive behavior. We found that reduced drinking behavior continued to be presented even when the transgene was expressed on an inbred strain background. These studies indicate the 5HT3 receptor plays a role in impulse control.

15. SUBJECT TERMS
5HT3 receptor, transgene, inbred mice, alcohol drinking,neurogenesis

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Introduction (Unmodified from original)

Alcohol use has been identified as an important factor in aggressive, or violent, behavior in humans. In its 10th Special Report to the U.S. Congress on Alcohol and Health (June 2000), the Public Health Service stated that one in four victims of violent crime described their attacker as having consumed alcohol prior to committing the crime. When the victim was a current or former intimate partner, the incidence increased to two out of three offenders having been drinking prior to the attack. Alcohol not only increases the incidence but also the severity of the attack. While these statistics may be correlational, a causal relationship between alcohol use and physical violence needs to be explored.

Experimentally, aggression in mice can be brought about by a isolating male for some time, then, subsequently, to introduce a group-housed male to the isolated male's home cage (*isolation-induced aggression or intruder-aggression*), with heightened attacks seen when the resident mouse is an actively breeding male (Malick, 1979; Miczek, 1999). Many environmental and experiential variables factor into aggressive behavior, but the administration of alcohol is an important societal variable in aggressive behavior (Miczek et al., 1998). Several lines of research on the neurochemical basis for aggression have implicated the serotonin system (Korte et al., 1996; Fish et al., 1999; DeAlmedia et al., in press). Alterations in the serotonin system, using knock out technology, have produced lines of mice that display altered aggressive behavior (Saudou et al., 1994; Cases et al., 1995). Pharmacologic studies have implicated the involvement of 5-HT_{1a} (deBoer et al., 2000), 5-HT_{1b} (Fish et al., 1999) and 5-HT_3 (Rudissaar et al., 1999) receptors. Several clinical studies have reported the observation that highly aggressive individuals display a serotonin-deficient trait, as measured by either low levels of cerebral spinal fluid (CSF) 5-hydoxyindole acetic acid (5-HIAA) or a flattened prolactin response to serotonin activation (Brown et al., 1982; Linnoila et al., 1983; Coccaro et al., 1990, Mann, 1999). While these data provide support for a role for serotonin in aggression, there is strong support for gamma amino butyric acid (GABA) as well (Soderpan and Svensson, 1999; Glaysheva et al., 1998; Guillot et al., 1998; Navarro and Pedraza, 1996; Miczek et al., 1995; 1994,1993; Weerts et al., 1993).

Besides the suggestion that serotonin plays a role in aggression, several lines of research have suggested a role for serotonin in the regulation of alcohol consumption. A number of studies indicate that the $5HT_3$ receptor system mediates alcohol consumption and the subjective effects of alcohol. $5HT_3$ receptor antagonists decrease alcohol intake in laboratory animals (Jankowska et al., 1995, Jankowska et al. 1994, Tomkins et al. 1995, Knapp and Pohorecky 1992, Hodge et al. 1993) and humans (Johnson et al., 1993; Sellers et al., 1994). In addition, $5HT_3$ receptor antagonists increase the subjective feeling of alcohol intoxication in humans (Swift et al., 1996), suggesting that $5HT_3$ receptors are involved in alcohol sensitivity. Lovinger and his colleagues have shown that alcohol directly potentiates the $5HT_3$ receptor (Lovinger, 1991; Lovinger and White, 1991; Lovinger and Zhou,

1994). The $5HT_3$ receptor is unique in the serotonin receptor family in that it is a cation channel and modulates the release of a number of other neurotransmitters, including GABA and dopamine. Thus, the $5\text{-}HT_3$ receptor is likely to play a critical role influencing alcohol consumption, which appears to involve dopamine and influencing both natural and alcohol-heightened aggression through the $GABA_A$ receptor system.

We hypothesized that over-expression of $5\text{-}HT_3$ receptors decreases alcohol consumption because the presence of an increased number of $5\text{-}HT_3$ receptors increased the potentiation of dopamine release at lower alcohol concentrations. Thus, the animal requires less alcohol to obtain the same behavioral effect. Alternatively, it is possible that the over-expression of $5\text{-}HT_3$ receptors increases the release of gamma aminobutyric acid (GABA), an inhibitory neurotransmitter. GABA plays an important role in the inhibitory circuit from the prefrontal cortex to the amygdala and ventral striatal areas. This pathway is thought to play a role in moderating impulsive and compulsive behaviors. Thus, the lower level of alcohol consumption seen in the $5\text{-}HT_3$ receptor over-expressing mice may be the result of increased inhibitory control over alcohol consumption. That is, these transgenic mice know “when to say when”. To add support for this hypothesis, we found that the $5\text{-}HT_3$ receptor over-expressing mice fail to behave aggressively in an intruder aggression test. The wild type mice clearly displayed high levels of aggression by attacking the intruder within seconds of introduction and continuing the attacks until the test was halted. This lower level of aggression in the $5\text{-}HT_3$ receptor over-expressing mice may be due to an increase in GABA activity. The proposed studies will explore the neurochemical relationship between the lower alcohol consumption and lower levels of aggression seen in $5\text{-}HT_3$ receptor over-expressing transgenic mice. The first goal will examine the impact of $5HT_3$ receptor over-expression on alcohol preference using a two-bottle free choice test. The second goal will examine the impact of $5HT_3$ receptor over-expression on natural aggressive behavior. Lastly, the impact of $5HT_3$ receptor over-expression on alcohol-heightened aggressive behavior will be measured.

Body

Progress made towards our goals are as follows: Breeding onto the three different backgrounds has been completed. Generations N1, N3 and N5 are the generations tested. We have additional data on impulse control in a novel object maze task as well as elevated plus maze behavior. The number of animals in each group ranged from 7 to 10. We have novel data on learned helplessness and neurogenesis in these mice and are planning work on using these findings to help develop a model for Post traumatic stress disorder and depression.

Results

B6SJL/F2-OE and C57Bl/6J-OE mice consume less ethanol while DBA/2J-OE do not.

Ethanol consumption was assessed for transgene positive and negative mice from the first (N1), third (N3) and fifth (N5) congenic generations. The effect of $5\text{-}HT_3$ receptor over-expression on

ethanol consumption depends on the strain in which it is expressed. In all generations tested, C57Bl/6J-OE mice display significantly reduced

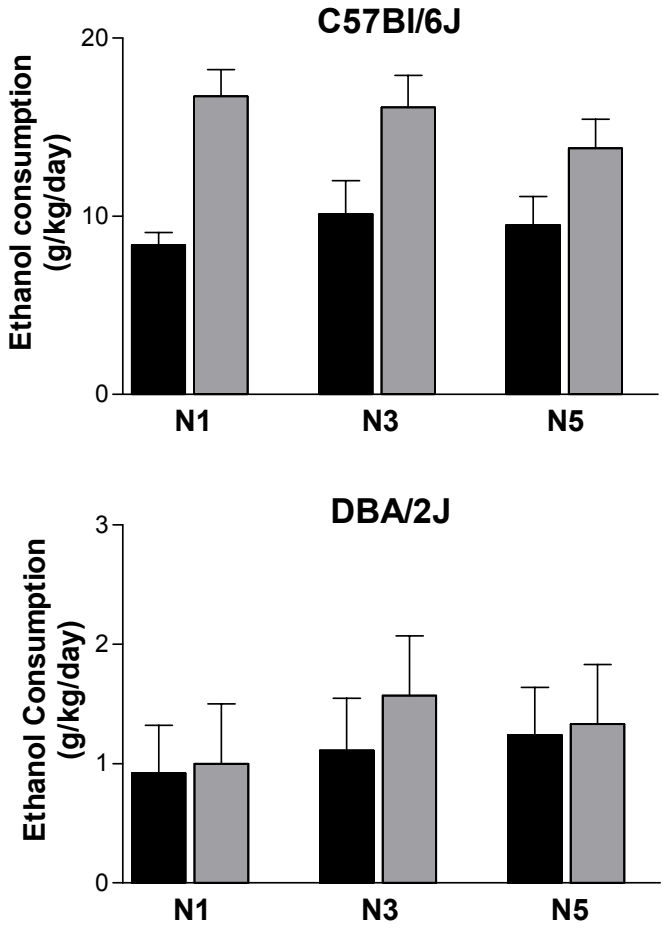
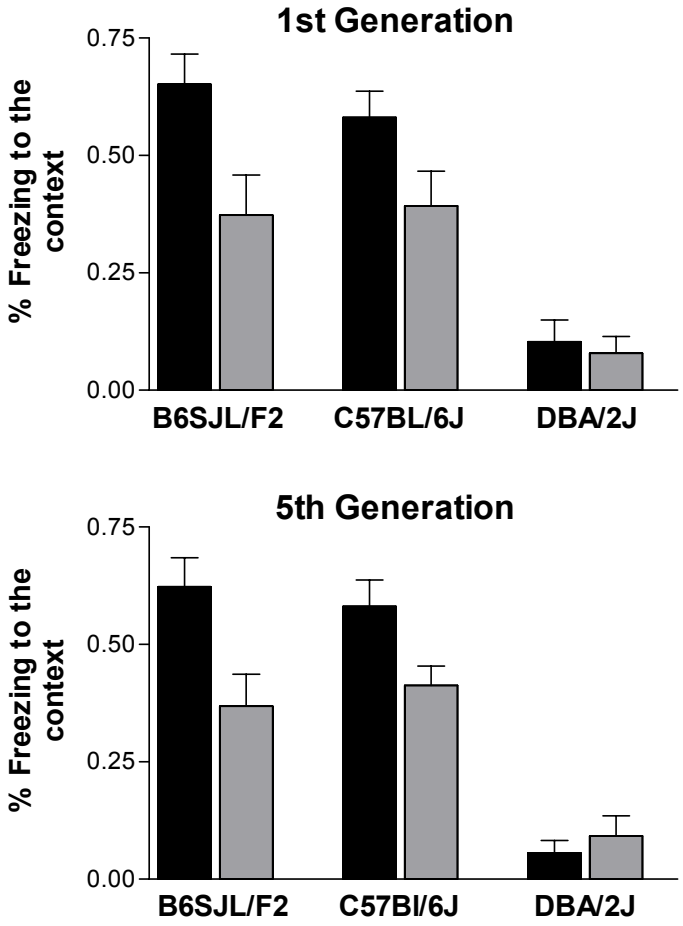


Figure 1. Ethanol consumption is influenced by 5-HT_3 receptor over-expression and background.

Data are AVG +/- SEM, (■) transgene positive, (▒) transgene negative. * indicates significant effect ($P < 0.05$) of transgene presence.

ethanol consumption compared to transgene negative mice of the same background (Figure 1A). However, a reduction in ethanol consumption was never noted for DBA/2J-OE mice (Figure 1B). DBA/2J mice, regardless of transgene presence, consume very low amounts of ethanol. A main effect of background was found for each N1 [$F(1,35) = 425.32$, $P < 0.0005$], N3 [$F(1,35) = 459.41$, $P < 0.0005$] and N5 generations [$F(1,35) = 349.24$, $P < 0.0005$]. A main effect of transgene presence was also found for each N1 [$F(1,35) = 56.0$, $P < 0.0005$], N3, [$F(1,35) = 33.85$, $P < 0.0005$] and N5 generations [$F(1,35) = 6.33$, $P < 0.017$]. Interactions of background and transgene presence were

found for N1 [F(1,35) = 54.50, p<0.0005], N3 [F(1,35) = 30.721, *P* < 0.0005] and N5 generations [F(1,35) = 4.35, *P* < 0.05].



B6SJL/F2-OE and C57Bl/6J-OE mice display improved contextual fear conditioning, whereas DBA/2J-OE mice do not.

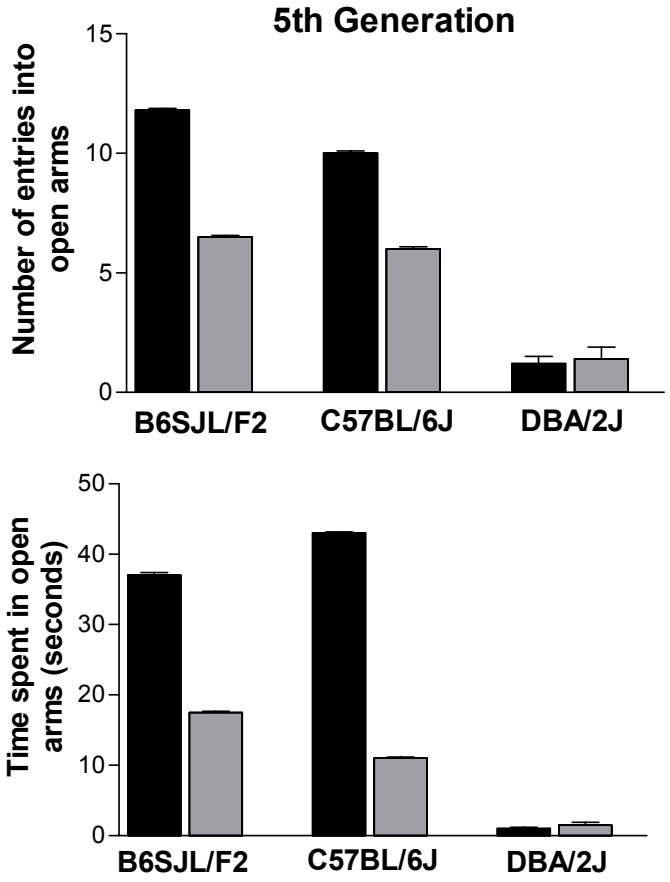
Fear conditioning to the context was determined for transgene positive and negative mice from N1 (Figure 2A) and N5 (Figure 2B) generations as well as B6SJL/F2 mice. None of the IS groups differed in freezing behavior and are not reported here. Transgene presence improved conditioning on B6SJL/F2 and C57Bl/6J backgrounds. A main effect of background was found for N1 [F(2,51) = 38.64, *P* < 0.0005] and N5 [F(2,51) = 16.15, *P* < 0.0005]. A main effect of transgene presence was also found for N1 [F(1,51) = 10.9, *P* < 0.003] and N5 [F(1,51) = 11.32, *P* < 0.001]. A background x transgene presence interaction was found for the N5 generation as well [F(2,51) = 4.55, *P* < 0.15].

Figure 2. Contextual conditioning is influenced by 5-HT$_3$ receptor over-expression and background.

Data are AVG +/- SEM, (■) transgene positive, (■) transgene negative. * indicates significant effect (*P* < 0.05) of transgene presence.

B6SJL/F2-OE and C57Bl/6J-OE, but not DBA/2J-OE mice, display an anxiolytic phenotype in the EPM.

Anxiety-related behaviors were assessed for transgene positive and negative mice from the N5 generations as well as B6SJL/F2 mice in the EPM. Number of entries into open arms (Figure 3A) and time spent in the open arms (Figure 3B) are reported here. Again, the transgene effect (anxiolysis) that had been previously observed was recapitulated using the B6SJL/F2 and C57Bl/6J but not the DBA/2J strain. For the measure of number of entries, main effects of background [F(2,51)



= 164.56, $P < 0.0005$] and transgene presence [F(1,51) = 51.66, $P < 0.0005$] were found, as was an interaction between background and transgene presence [F(2,51) = 13.93, $P < 0.0005$]. For the measure of percent time spent in open arms, main effects of background [F(2,51) = 113.64, $P < 0.0005$] and transgene presence [F(1,51) = 103.81 $P < 0.0005$] were found. An interaction of background and transgene presence was detected [F(2,51) = 31.667, $P < 0.0005$].

Figure 3. Anxiety measures in the EPM are affected by 5-HT_3 receptor over-expression and background.

Data are AVG +/- SEM, (■) transgene positive, (■) transgene negative. * indicates significant effect ($P < 0.05$) of transgene presence.

Both C57Bl/6J-OE and DBA/2J-OE spend more time near a novel object.

Inquisitive and inspective behaviors were assessed in an open area after the insertion of a novel object for transgene positive and negative mice from the N5 generations, as well as B6SJL/F2 mice. As total number of lines crossed and latency to approach the novel object did not differ between groups, only the total number of entries into the center (Figure 4A) and time spent near the novel object (Figure 4B) are reported here. For the number of entries into the center area where the novel object was placed, an effect of transgene presence was found for the C57Bl/6J but not the DBA/2J background (Figure 4A). Surprisingly, in the measure of time spent near the novel object, transgene presence had an effect for both C57Bl/6J and DBA/2J backgrounds (Figure 4B). For the measure of total entries into the center area, main effects of background [$F(2.51) = 35.0$, $P < 0.0005$] and genotype [$F(1,51) = 20.311$, $P < 0.0005$] as well as an interaction between background and transgene presence [$F(2,51) = 3.63$, $P < 0.05$] were found. Percent time spent near the novel object was influenced by background [$F(2.51) = 17.95$, $P < 0.0005$] and transgene presence [$F(1,51) = 708.82$, $P< 0.0005$].

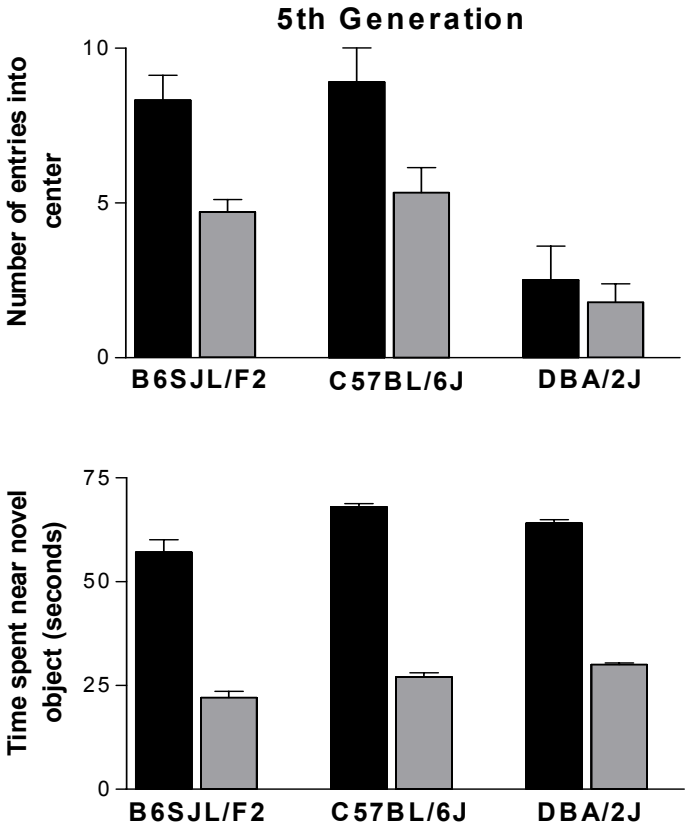


Figure 4. Inspection of a novel object is enhanced by 5-HT_3 receptor over-expression regardless of background.

Data are AVG +/- SEM, (■) transgene positive, (■) transgene negative. * indicates significant effect ($P < 0.05$) of transgene presence

DISCUSSION

The behavioral phenotype of C57Bl/6J-OE mice supports the original findings in B6SJL/F2-OE mice. Ethanol consumption was reduced, contextual fear conditioning was improved, a reduction in anxiety was indicated using the EPM, and inspective behavior toward the novel object was increased. DBA/2J-OE mice did not show any of these pleiotropic effects of transgene expression, save for an increase in the amount of time spent near the novel object, a measure of inspective behavior (Grailhe *et al.* 1999). The reason for this overall lack of effect of transgene expression on the DBA/2J background may be due to differences in intracellular signaling directly influencing 5-HT_3 receptor activity, or alternately due to differences related to dopamine as a consequence of 5-HT_3 receptor over-expression

Ethanol consumption is one of the most stable behaviors in mice (Crabbe *et al.* 1999), and so was observed in all three N1, N3, and N5 congenic generations to follow the effect of the transgene over-time. The effect of background strain was stable over all generations (Figure 1). One reason that DBA/2J-OE mice do not show reduction in ethanol consumption could be a floor-effect. DBA mice drink very little regardless of transgene presence so it may not be possible for this strain to consume any less alcohol, especially given that the bottle position is changed daily, and any attempt to avoid ethanol consumption, if found aversive, would still result in low levels of drinking similar to levels observed. We previously reported that ethanol is not rewarding, and indeed may be aversive, at higher doses for B6SJL/F2-OE (Engel & Allan 1999; Engel *et al.* 1998). The data presented here support this effect of 5-HT_3 receptor over-expression. Furthermore, this experiment demonstrates the critical impact that background choice can have on behavior. The use of C57Bl/6J mice, and not DBA/2J, is advised when a reduction in ethanol consumption is expected as a consequence of genetic manipulation.

Over-expression of the 5-HT_3 receptor improved contextual fear conditioning on the B6SJL/F2 and C57Bl/6J backgrounds but not the DBA/2J. As DBA/2J mice have poor contextual fear conditioning (Paylor *et al.* 1994), it was speculated that improvements in contextual conditioning would be most evident on this background. Reduced PKC activity, previously reported in the hippocampus of DBA/2J mice (Bowers *et al.* 1995; Wehner *et al.* 1990) may explain the lack of effect of the transgene on hippocampal-dependent learning. 5-HT_3 receptors in Xenopus oocytes are potentiated by PKC activation (Sun *et al.* 2003; Zhang *et al.* 1995). Thus, DBA/2J mice may have reduced potentiation of the 5-HT_3 receptor, even when over-expressed. As potentiation of the 5-HT_3 receptor is proposed to contribute to the reduction in ethanol consumption (10), and may also underlie enhanced learning and memory in B6SJL/F2-OE mice, the DBA/2J PKC activity deficit may preclude potentiation of the 5-HT_3 receptor. While it has been proposed that DBA/2J mice could provide an appropriate genetic background to study enhancement of hippocampal-dependent learning and memory, this strain's usefulness may be limited due to an intrinsic deficit in intracellular signaling. We propose that molecular manipulations aimed at improving hippocampal-dependent learning that may be mediated in part by PKC activity, such as 5-HT_3 receptor over-expression, might not be able to overcome the learning deficit of the DBA/2J strain.

Alternately, the lack of improvement in contextual conditioning noted for DBA/2J mice could be due to differences in nucleus accumbens (NAc) function in this task. Inactivation of the NAc selectively reduces contextual fear conditioning (Haralambous & Westbrook 1999; Riedel *et al.* 1997; Westbrook *et al.* 1997). Additionally, NAc function appears to vary in relation to a strains' propensity to develop contextual conditioning (Ammassari-Teule *et al.* 2000). As the 5-HT_3 receptor modulates dopamine release in the NAc (Allan *et al.* 2001; Campbell & McBride 1995; Jiang *et al.* 1990; Sung *et al.* 2000; Wozniak *et al.* 1990), it is possible that over-expression modulates conditioning in a strain

dependent manner. Dopamine release has been implicated in reward processing, but is also affected by aversive conditions, such as fear conditioning, and may influence learning (Horvitz 2000, 2002; Schultz 2002). Therefore, differences in NAc activity between strains may influence behaviors altered by 5-HT_3 receptor over-expression.

Anxiety, as measured in the EPM, also remained unchanged by 5-HT_3 receptor over-expression on the DBA/2J background, whereas B6SJL/F2-OE and C57Bl/6J-OE displayed reduced anxiety in the EPM. The reason for this is not clear. Both DBA/2J and C57Bl/6J strains are emotionally reactive in the EPM (Griebel *et al.* 2000), indicating that 5-HT_3 receptor over-expression should affect both similarly. While fearful reactions are generally considered to be controlled by the amygdala (Davis & Whalen 2001; Rogan & LeDoux 1996), the hippocampal formation is also important (File *et al.* 2000; Gonzalez *et al.* 1998). In fact, the hippocampal formation is thought to be capable of coding critical aspects of anxiety, allowing for the integration of anxiety states and learning cues. It is therefore possible that hippocampal deficits, noted for DBA/2J mice, underlie the inability of 5-HT_3 receptor over-expression to modify anxiety related behaviors in this strain.

The one task where transgene presence produced a change in behavior on all backgrounds was the novelty exploration task. Time spent near the novel object was increased for all three strains. However, total number of entries into the center area near the novel object was not increased in DBA/2J-OE mice. This could be influenced by heightened anxiety-related behavior in DBA/2J-OE mice relative to C57Bl/6J-OE and B6SJL/F2-OE mice. It is possible that there is some threshold related to anxiety that influences the number of entries into the center. However, once this threshold is crossed and the animal is in the center near the novel object, exploration of the object reflected by time spent near it, is enhanced as a consequence of over-expression of the 5-HT_3 receptor..

The exploration of a novel object may depend on dopamine, the release of which is potentiated in 5-HT_3 receptor over-expressing mice (Allan *et al.* 2001; Sung *et al.* 2000). In human brain, the ventral striatum (NAc) responds to novelty in the absence of awareness (Berns *et al.* 1997). Furthermore, heightened exploratory behavior in a novel environment predicts a greater DA response in the NAc (Hooks *et al.* 1991). This suggests that greater dopamine release in the NAc, mediated by activity at over-expressed 5-HT_3 receptors, may influence the propensity to explore novel objects or environments.

One final consideration is that maternal behavior could influence the ability of the transgene to affect phenotype. A nongenomic behavioral mode of inheritance from parent to offspring has been demonstrated in rat. More attentive female rats, characterized by licking, grooming and arched-back nursing, yield dams with reduced fearfulness regardless of parental genotype (Francis *et al.* 1999). Furthermore, dams raised by highly attentive female rats have changes in mRNA in regions such as the hippocampus and amygdala, demonstrating that expression of genes can be influenced by maternal behavior (Francis *et al.* 1999). This raises the possibility that the behavioral profile of 5-HT_3

receptor over-expressing animals may be further influenced by nongenomic factors. In conclusion, our results underscore the need to consider the genetic environment conferred by strain selection on the effects of genetic manipulation in mice.

New Information for 2005

Learned helplessness and neurogenesis in 5HT3 OE mice

Learning and exposure to a novel environment enhance, while stress, and perhaps depression, reduces, neurogenesis in the adult dentate gyrus (DG) of the hippocampus. Transgenic mice over-expressing the 5-HT3 receptor for serotonin (5-HT3-OE mice) display improvements in hippocampal-dependent learning, heightened inspective behavior towards novel objects, and are less anxious. To assess the correlation of changes in neurogenesis with depression-like behaviors in 5-HT3-OE mice, proliferation, survival and differentiation were quantified in the DG and depression-like behavior was examined using the forced swim test (FST) and learned helplessness (LH) paradigms.

Cells born in the hippocampus of adult 5-HT3-OE and wild-type littermates (WT mice) were labeled with 5-bromo-2'-deoxyuridine (BrdU) by intraperitoneal injection (IP) or subcutaneous (SQ) infusion via mini-osmotic pump. 5-HT3-OE mice had reduced proliferation and an increase in survival compared to wild-type (WT). BrdU delivery method had a significant effect on differentiation, with injection resulting in an increase in the number of BrdU+ cells of undetermined phenotype. 5-HT3-OE mice also demonstrated an antidepressant-like phenotype in both FST and LH tests. Repeated injection of BrdU may not necessarily reflect basal neurogenesis. Rather, this method of labeling new cells may alter the phenomenon being observed. Results indicate that 5-HT3-OE mice have enhanced survival in the face of reduced proliferation – in effect, enhanced neurogenic efficacy. This may contribute to an antidepressant-like phenotype.

NEUROGENESIS EXPERIMENTS

PROGENITOR STUDY

10 (5-HT3-OE) and 14 (WT) mice were given IP injections of 300 mg/kg BrdU at 4 time-points (0, 4, 8, and 12 hours) and were sacrificed at 24 hours (Figure 1A). This BrdU regimen labels the entire proliferative population in C57Bl/6J mice (10), which have the highest observed rate of proliferation of all mouse strains studied (9). BrdU labeling in the DG of the hippocampus was assessed using immunohistochemistry, From the same pool of animals, 6 animals of each genotype were assessed for BrdU labeling in the SVZ.

PROLIFERATION STUDY

Groups consisted of 8 animals x 2 genotypes. Animals were given an IP injection of 300 mg/kg BrdU twice daily, 8 hours apart, every other day (days 0, 2, 4, and 6) and were sacrificed on day 7 (Figure 2A). Labeling on multiple days was used to increase the number of labeled cells and to reduce

the effects of label dilution with continued proliferation, as previously described (3, 5, 8, 42). BrdU labeling in the DG was assessed using immunohistochemistry.

MINI-OSMOTIC PUMP STUDY

Groups consisted of 8 animals x 2 genotypes x 2 delivery methods x 2 sacrifice time-points, except that one mouse was removed from the WT injected control group sacrificed at 7 days due to observed 'repetitive circling' behavior. Animals were analyzed in the four resulting groups, pump-implanted 7 day time-point (P7), injected controls 7 day time-point (J7), pump-implanted 28 day time-point (P28), and injected controls 28 day time-point (J28). P7 and J7 groups were processed in parallel, as were P28 and J28. Mini-osmotic pumps delivered 0.638 mg BrdU SQ daily and injected controls received 7.5 mg BrdU IP daily (See Materials). Injections and pump infusion lasted 7 days at which point half of the animals were sacrificed. The other half were sacrificed at 28 days (Figure 3A).

IMMUNOHISTOCHEMISTRY

Animals were killed with an overdose of sodium pentobarbital followed by perfusion With 4% paraformaldehyde (PFA). Brains were removed from the skulls, postfixed for 1 day in PFA, then cryoprotected in 30% sucrose for 2 days and frozen. Serial coronal 30 □m sections of the hippocampus were cut from Bregma -1.2mm to Bregma -3.0mm of either left or right hemisphere using a cryostat. Every sixth section was mounted onto a coded slide. BrdU+ cells in the DG were counted and summed over 9 total sections per mouse. For the progenitor study, 6 sections for each mouse were taken in the same manner from the striatum to visualize new cell birth in the SVZ. Briefly, sections were permeabilized in 0.4% triton-X in saline followed by 2N HCL treatment at 45ºC. Sections were rinsed in 0.1M Borate Buffer, blocked with 5% Donkey serum followed by overnight incubation at 4ºC with primary antibody. 24 hours later the sections were rinsed and incubated overnight at 4ºC with secondary antibody, and the next day the sections were rinsed and coverslipped. For progenitor and proliferation studies (7 day sacrifice time-points), only primary rat anti-BrdU followed by secondary Texas Red-conjugated donkey anti-rat were used to visualize BrdU+ cells. Rat anti-BrdU, rabbit anti-GFAP and mouse anti-NeuN, as well as the correlated secondary antibodies were used for triple labeling at the 28 day time-point in the mini-osmotic pump study. BrdU+ cells were counted at 40x using fluorescent microscopy. In the DG, cells were counted within the granule cell layer, defined as within 2 cell body widths of this cell layer. Cell counts from the 9 sections were combined and are reported as the number of BrdU+ cells in the DG. This number represents a sample of the total population and this study does not attempt to quantify the absolute number of BrdU+ cells within the DG of 5-HT3-OE and WT mice. For detection of co-labeled cells at the 28 day time-point, z-stacks were taken using a Zeiss laser scanning confocal microscope (Zeiss LSM 510) using a 63x objective. Fifty BrdU positive cells were analyzed per animal for verification of colocalization by optically slicing 0.5 □m intervals in the Z plane.

DEPRESSION TESTS

LEARNED HELPLESSNESS

Apparatus– A Coulbourn Habitest Shuttlebox with a stainless steel grid floor for administration of the 0.3 mA footshock was used for training and testing. The front and back were made of Plexiglas. The sides, including a dividing guillotine door in the center, and the ceiling were aluminum. The apparatus was located within a sound-attenuated chamber. 70% ethanol was used to clean the walls and floor after the removal of each mouse from the shuttlebox during training and testing.

Training – Groups consisted of 8 animals x 2 genotypes x 2 training groups. Animals in the learned helplessness (LH-trained) group received 120 uncontrollable and unpredictable footshocks (0.3mA, 6 second duration) over the course of one hour. A probability for delivery of the footshock was assigned at 0.5 every 15 seconds and the animal was removed 30 seconds after the delivery of 120 shocks. The non-shocked (NS-trained) group was placed into the apparatus and given an hour exposure without any shock being delivered. Mice from each training group were removed at the end of the hour and allowed 24 hours before being tested. Two mice were trained at a time, one LH-trained and one NS-trained. Only animals caged together, and therefor of the same gender, were trained at the same time.

Testing – To test for LH, an escape deficit was measured during an active avoidance procedure. Animals were placed into the shuttlebox and given 30 trials with an intertrial interval of 30 seconds. For the first 5 trials, a footshock and auditory cue (80 db, 6 Hz clicker) came on at the start of the trial and, and after a 1-second delay the guillotine door raised. The last 25 trials initiated with the auditory cue and the door raising, the footshock was delayed for 3 seconds. Latency for the mouse to escape through the door was measured as the time from the door raising to the time it closed. Sensors in each compartment of the shuttlebox turned off the footshock and tone and closed the guillotine door if the animal escaped through the raised door. If no escape was made 24 seconds after the start of the trial, the shock and tone terminated and the door closed. Not all animals developed learned helplessness regardless of genotype. Criteria for developing helplessness was set at 50% failure to escape footshock during testing over the first 10 trials. Two LH-trained animals did not reach criteria from both the WT and 5-HT3-OE groups. Final groups consisted of 8 animals.

FORCED SWIM TEST

15 animals from each genotype were tested. Mice were placed in a 30 cm diameter, 46 cm tall cylinder of water (22-25°C, depth 26 cm) and forced to swim for 6 minutes as described previously (43, 44). Six behaviors were scored as described by Scramm et al. (45). 1=floating,

2=twitching, 3=kicking, 4=thrashing, 5=climbing, and 6=swimming behaviors (Figure 5C) were assessed every 5 seconds. The combination of floating, kicking and twitching behaviors represents immobility indicative of depression-like behavior. So, the sum of swimming, climbing and thrashing behavior was tallied and expressed as a percentage of total observations to describe escape-directed behavior during the last 4 minutes.

ANALYSIS

In all cases, an initial Analysis of Variance (ANOVA) was performed with hemisphere as a factor. No differences were noted between left and right hemispheres in any of the studies so all data were collapsed across hemispheres. Two-way ANOVAs (genotype x gender) were used for analysis of BrdU+ cells in the Progenitor Study and the Proliferation Study and for the analysis of escape-directed behaviors in the FST. For the Mini-Osmotic Pump Study, two-way ANOVAs (genotype x gender) were also used for the analysis of BrdU+ cells for each of the 4 groups, P7, J7, P28, and J28. Survival was compared in the Mini-Osmotic Pump Study using a three-way ANOVA (genotype x delivery method x sacrifice time-point) after gender was found not to be a factor. To assess differentiation into neurons (NeuN+) and glia (GFAP+), BrdU labeled cells positive for either or no marker in the Mini-Osmotic Pump Study at the 28 day time-point (P28 and J28 groups) were analyzed using a two-way ANOVA (genotype x delivery method) after no effect of gender was found. For LH, a two-way ANOVA (genotype x testing bin) was used to analyze the LH-trained group after no effect of gender was found, and a three-way ANOVA (gender x genotype x testing bin) was used for the NS-trained group for LH.

New RESULTS

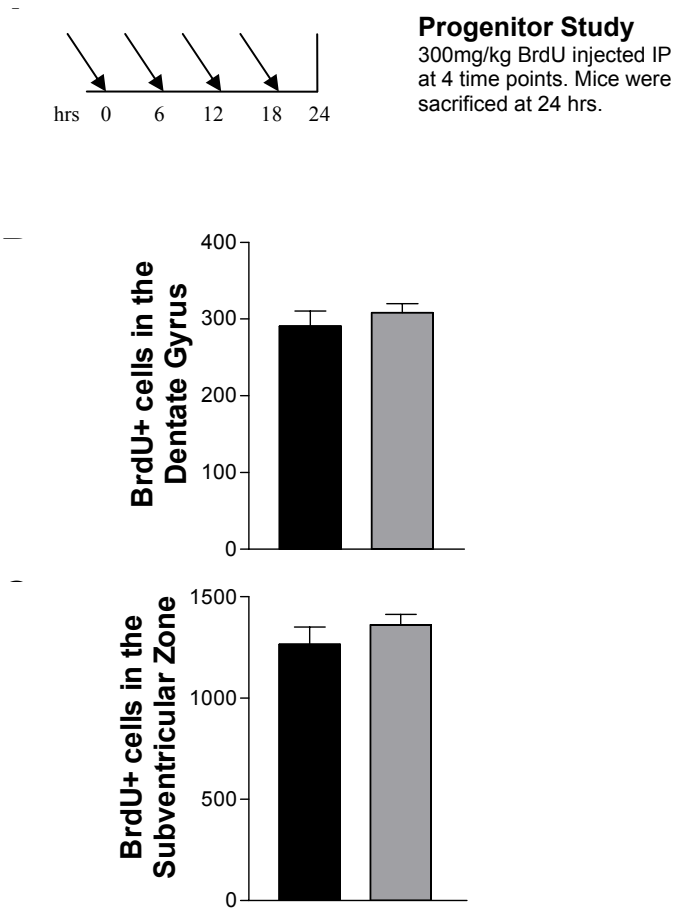


Figure 1. The number of BrdU+ cells produced within 24 hours in the DG and SVZ is not changed by 5-HT$_3$ receptor over-expression.

Data are Avg ± SEM, (■) 5-HT$_3$-OE, (▒) WT. Study design (A). n=10 5-HT$_3$-OE and n=14 WT (B), n=6 (C). 5-HT$_3$-OE mice are not different from WT in number of progenitors detected in either the DG (B) or SVZ (C).

NEUROGENESIS

The number of cells incorporating BrdU within 24 hours is not different between 5-HT3-OE and WT mice in either the DG or SVZ.

To identify cells capable of mitosis and their daughter cells (called progenitors here) in a 24-hour period, the DG and SVZ were examined for cells incorporating BrdU following four IP injections of 300 mg/kg BrdU (Figure 1A). The number of labeled cells in the DG (Figure 1B) and SVZ (Figure 1C) was not affected by 5-HT3 receptor over-expression. For the DG, a two-way ANOVA (genotype x gender) revealed no effect of genotype, but an effect of gender [$F(1,20) = 8.084$, $P < 0.01$]. Data

suggest a slight reduction in the number of dividing cells in the DG of female mice compared to male. In the SVZ, a two-way ANOVA (genotype x gender) revealed no main effects or interactions.

5-HT3-OE mice have reduced proliferation in the DG compared to WT at 7 days regardless of BrdU delivery method.

BrdU labeled cells were counted in the DG after 7 days of repeated BrdU injection. As shown in Figure 2A, Animals were injected with 300 mg/kg BrdU twice on days 0, 2, 4, and 6, then sacrificed on day 7 to provide an index of proliferation. A two-way ANOVA (genotype x gender) revealed only a main effect of genotype [$F(1,12) = 20.308$, $P < 0.001$]. Data presented n Figure 2B show a significant reduction in proliferation for 5-HT3-OE mice.

To assess proliferation by a different method, 5-HT3-OE and WT mice were divided into two groups, pump implanted mice (P) and injection control mice (J). Half were sacrificed at 7 days (P7 and J7), the other at 28 days (P28 and J28) as shown in Figure 3A. Mini-osmotic pumps from Alzet (Durect Corporation) were filled with 25 mg/ml BrdU in 37ºC saline (pumping rate of 1.06ul/hr or 0.636 mg BrdU/day), and implanted subcutaneously (SQ) under light halothane anesthesia. An injected control group was given daily IP injections (200 □l, or 5.0 mg BrdU per day) of the same 25 mg/ml, 37ºC BrdU solution. Proliferation at 7 days was reduced in 5-HT3-OE mice compared to WT for both pump implanted (Figure 3B) and injected control (Figure 3C) mice. A main effect of genotype for the P7 group was found using a two-way ANOVA (genotype x gender) [$F(1,12) = 5.208$, $P < 0.05$] (Figure 3B). The J7 group showed the same main effect of genotype [$F(1,11) = 46.516$, $P < 0.0005$] (Figure 3C). An effect of gender [$F(1,11) = 7.072$, $P < 0.05$] was also found for the J7 group. Females of both genotypes had slightly reduced proliferation compared to males when repeated IP injection of BrdU was used to label dividing cells.

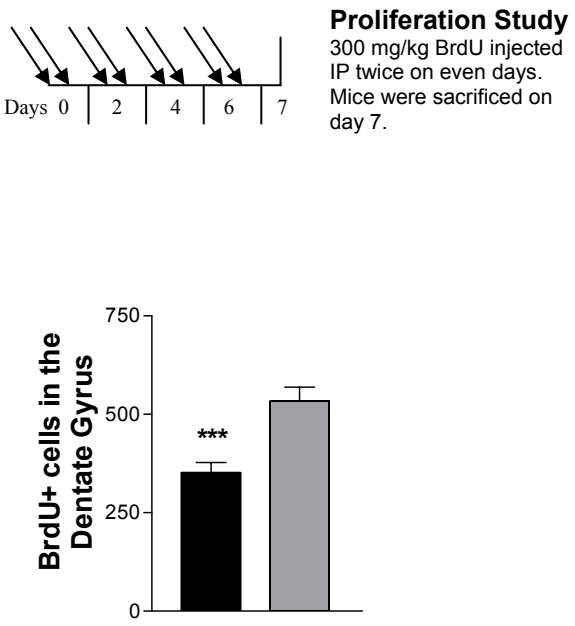


Figure 2. 5-HT$_3$-OE mice have fewer BrdU+ cells in the DG 7 days after repeated BrdU injection.

Data are Avg ± SEM, (■) 5-HT$_3$-OE, (▒) WT. n=8. *** p<0.001. Study design (A). 5-HT$_3$-OE mice have reduced numbers of BrdU+ cells in the DG at 7 days compared to WT (B).

5-HT3-OE mice have enhanced survival compared to WT regardless of delivery method.

P28 and J28 5-HT3-OE and WT mice were sacrificed at 28 days to assess the number of BrdU+ cells in the DG. Data indicate no difference in the number of BrdU+ cells for P28 (Figure 3B) or J28 (Figure 3C) groups. Differences in survival were assessed by combining the data from both the 7 and 28 day sacrifice time-points. Data show that BrdU delivered via SQ mini-osmotic pump (Figure 3B) labeled more cells than IP injection (Figure 3C). 5-HT3-OE mice sacrificed at 7 days had reduced proliferation while the number of new cells labeled at 28 days was not different from WT. This is shown as enhanced percentage of survival in Figure 3D. The percentage of cells surviving for the P28 group (100% x (P28 avg □ P7 avg) was 57.4% for 5-HT3-OE mice, and 46.4% for WT. Survival for the J28 group (100% x (J28 avg □ J7 avg)) was 49.3% for 5-HT3-OE mice, and 36.9% for WT. A three-way ANOVA (genotype x delivery method x sacrifice time-point) revealed main effects of delivery method [$F(1,55) = 68.64$, $P < 0.0005$], sacrifice time-point [$F(1,55) = 117.49$, $P < 0.0005$], and genotype [$F(1,55) = 19.55$, $P < 0.0005$]. Additionally, an interaction between genotype and sacrifice time-point was found [$F(1,55) = 10.06$, $P < 0.005$].

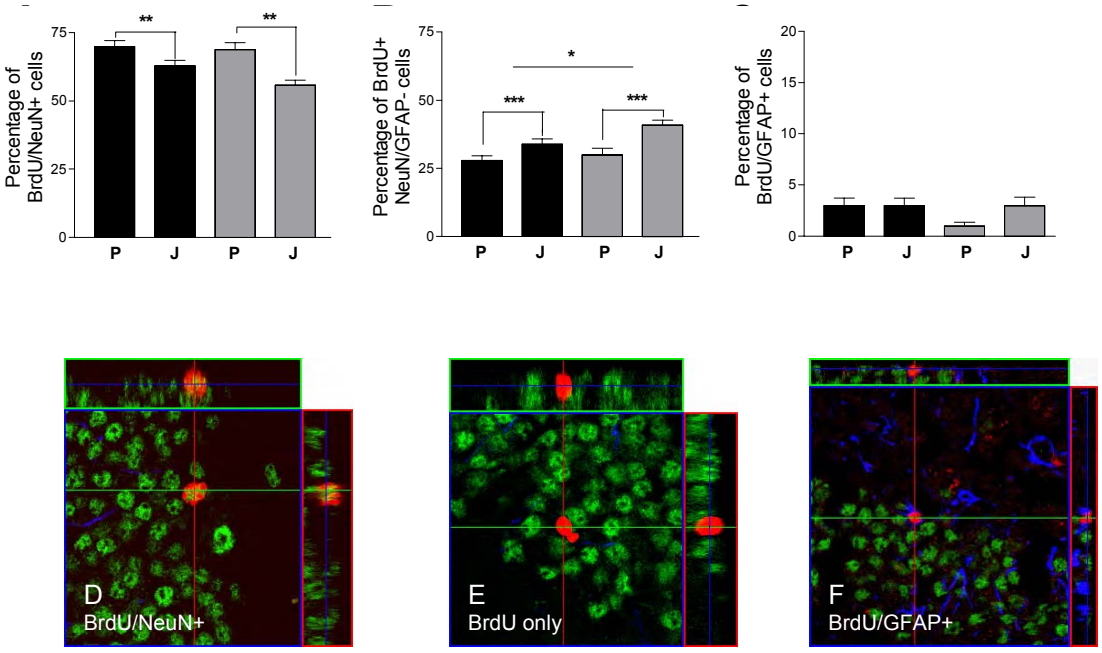


Figure 4. Differentiation is affected by 5-HT$_3$ receptor over-expression and BrdU delivery method.

Data are Avg ± SEM, (■) 5-HT$_3$-OE, (▒) WT. n=8. P indicates pump-implanted animals, J injected controls sacrificed at 28 days. * $p<0.05$, ** $p<0.001$, and *** $p<0.0005$. Compared to pump infusion of BrdU, injection reduces the percentage of mature BrdU/NeuN+ new neurons (A) and increases the percentage of BrdU+, NeuN/GFAP- new cells (B) in the DG of both WT and 5-HT$_3$-OE mice. A genotype effect indicates WT mice have more BrdU+, NeuN/GFAP- new cells in the DG than 5-HT$_3$-OE mice regardless of delivery method (B). The percentage of BrdU/GFAP+ cells is unaffected by genotype or delivery method (C). Orthogonal projections, original magnification 63x, (D-F) from confocal z-stacks (0.5um interval) showing a BrdU/NeuN+ double-labeled neuron (D), a BrdU+, NeuN/GFAP- cell (E), and a BrdU/GFAP+ double-labeled cell in the DG (F).

Differentiation is effected by genotype as well as BrdU delivery method.

BrdU labeled cells were assessed for co-labeling with a mature neuronal marker, NeuN, and an astroglial marker, GFAP. Data in Figure 4A show a reduction in the number of new neurons when using the injection delivery method. WT mice have significantly elevated BrdU+ cells of undetermined phenotype (NeuN/GFAP-) using either delivery method, as shown in Figure 4B. Additionally, IP injection of BrdU increases the number of BrdU+ cells of undetermined phenotype compared to pump infusion (Figure 4B). BrdU/GFAP+ double-labeled cells were not different between groups as shown in Figure 4C. Differentiation was analyzed for P28 and J28 groups collapsed across gender using a two-way ANOVA (genotype x delivery method). For the percentage of BrdU/NeuN+ double-labeled neurons, there was no significant effect of genotype. A main effect of delivery method was found [$F(1,28) = 13.064$, $P < 0.001$]. Additionally, for the percentage of BrdU+ cells of undetermined phenotype, a main effect of genotype [$F(1,28) = 5.01$, $P < 0.05$], and delivery method [$F(1,28) =$

20.026, P < 0.0005], was revealed. No main effects or interactions were noted for the BrdU/GFAP+ double-labeled cells.

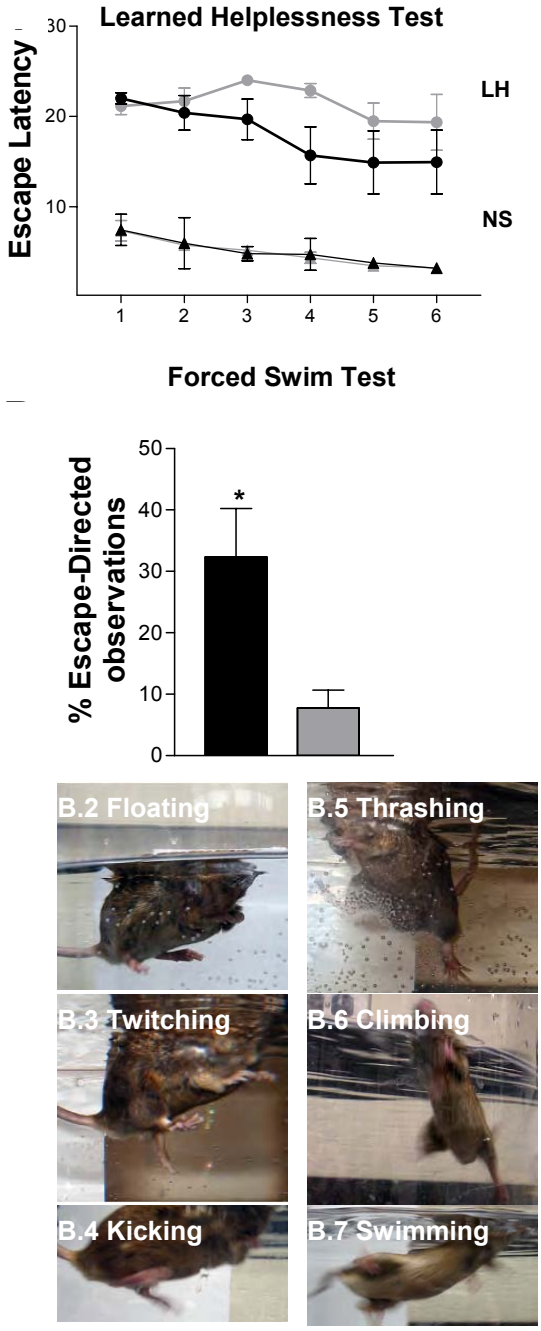


Figure 5. 5-HT$_3$-OE mice display antidepressant-like behavior.
Data are Avg ± SEM, (●,▲,■)5-HT$_3$-OE, (●,▲,■) WT. n=8 (A) n=15 (B). * p<0.05. LH-trained 5-HT$_3$-OE mice (●) have significantly reduced latency to escape (p<0.01) in the learned helplessness paradigm when compared to WT (●) indicating an antidepressant-like effect (A). The NS-trained WT (▲) and 5-HT$_3$-OE (▲) groups are not different (A). 5-HT$_3$-OE (■) mice have an antidepressant-like phenotype in the FST as shown by an increase in escape directed behaviors compared to WT (■) (B.1). Escape-directed behaviors (B.2-B.4) and immobility behaviors (B.5-B.7).

DEPRESSION TESTS

5-HT3-OE mice display an antidepressant phenotype.

5-HT3-OE display a decrease in escape latency during the LH active avoidance test, as shown in Figure 5A, and an increase in escape-directed behaviors in the FST, as shown in Figure 5B. In the active avoidance test for the LH-trained groups, no main effect of gender was found so data were collapsed across gender and a two-way ANOVA (genotype x bin) revealed a significant effect of genotype [$F(1,84) = 7.21$, $P < 0.01$]. A three-way ANOVA for the NS-trained groups (genotype x gender x bin) revealed no significant effect of genotype. However, a main effect of gender [$F(1,72) = 4.27$, $P < 0.05$], as well as a genotype x gender interaction was found [$F(1,72) = 12.60$, $P < 0.001$], A main effect of bin [$F(5,72) = 5.17$, $P < 0.0005$] was also noted. In the FST, a two-way ANOVA (genotype x gender) indicated a main effect of genotype [$F(1,26) = 6.634$, $P < 0.02$], while no main effect of gender was noted and no interactions were found between genotype and gender.

Discussion of New findings

The results of these studies demonstrate that, while 5-HT3-OE mice have reduced proliferation compared to WT, increased neurogenic efficacy is evidenced by an improvement in survival of newly generated cells in the DG. For pump implanted and injected control animals, 5-HT3 receptor over-expression resulted in increased survival, 24% and 34% over that of WT, respectively. Even though a non-significant increase was noted for 5-HT3-OE mice in terms of BrdU/NeuN+ double-labeled cells, the hypothesis that 5-HT3-OE mice would produce significantly more new neurons was not supported. Although serotonin and antidepressants have been shown to enhance proliferation and neurogenesis (17, 22), our results demonstrate that over-expression of the 5-HT3 receptor does not enhance basal neurogenesis. It would be interesting to see if enhanced survival confers resistance to stress-induced decreases in neurogenesis in 5-HT3-OE mice. 5-HT3-OE mice demonstrate an antidepressant-like phenotype, as hypothesized, and this correlates with enhanced survival of new cells in the DG.

The Progenitor Study assessed new cell birth at 24 hours. The protocol that we employed has been shown to label all cells capable of mitosis within a 24 hour period (10), and thus was used to determine whether 5-HT3-OE mice were different from WT in the number of cells capable of mitosis. The results indicate that bioavailability of BrdU is not likely different due to transgene expression as both the SVZ and DG had similar numbers of new cells labeled, regardless of genotype. 5-HT3-OE and WT mice do not differ in the number of progenitor cells seeding the SVZ and the DG, as shown in Figure 1. This suggests that differences in proliferation noted for 5-HT3-OE mice at 7 days (discussed below) are not likely due to a decrease in the number of progenitor cells capable of mitosis, but rather a decrease in observable proliferation as a consequence of changes in cell-cycle, or perhaps cell death. Although the maximal period of cell death is typically between 1 and 2 weeks after cell birth (2-5), some cell death likely occurs within the first week. Given that a portion of labeled cells incorporate BrdU on the terminal division, and are thus further along the path of neurogenesis when labeled, it is

likely that cell death claims a number of new born cells within the first week of the labeling protocol used to assess proliferation. Thus, increased cell death would appear as reduced proliferation at the 7 day sacrifice time point. Though if this were the case, then 5-HT3-OE mice, which have fewer labeled new neurons at 7 days, would have increased cell death, and thus reduced survival. Paradoxically, 5-HT3-OE mice were found to have enhanced survival, and presumably reduced cell death, using two delivery methods. One potential explanation to reconcile the 24 hour and 7 day BrdU cell counts is that while producing the same number of new cells, 5-HT3-OE mice have cell death occurring earlier than WT mice.

When sacrificed at 7 days, 5-HT3-OE mice had reduced proliferation compared to WT using all three methods, whether IP injection or SQ pump infusion. A range of BrdU doses were used for these studies, from approximately 0.64 mg/day for the pump implanted animals, to 5.0 mg/day for the injected controls of the Mini-Osmotic Pump Study and approximately 7.5 mg/day (300mg/kg for a 25g mouse) in the original Proliferation Study. The reproducibility of proliferation results across delivery methods and BrdU dosages then seems independent of any potential toxic effects of BrdU, which are not expected at the doses we used. Doses as high as 600 mg/kg have no deleterious effects in adult rats (42). If BrdU is toxic it is not differentially so as both 5-HT3-OE and WT groups retain their relationship (5-HT3-OE proliferation reduced relative to WT) across a range of concentrations of BrdU when assessed at 7 days.

If the 7 day time point truly reflects proliferation, the explanation for reduced proliferation after 7 days in the DG of 5-HT3-OE mice is not clear. Stress negatively impacts proliferation (53). Additionally, cell proliferation in the DG of adult rats highly reactive (negatively) to novelty is reduced (54). As 5-HT3-OE mice are less anxious in the elevated-plus maze, have an inspective, or positive, reaction to novelty (39) and are resistant to stress-induced depression, as reported here, it seems unlikely that a heightened stress response would be responsible for the reduced proliferation noted after 7 days. As changes in proliferation do not always result in concomitant changes in neurogenesis (55), the behavioral phenotype of 5-HT3-OE mice may be supported by enhanced survival of new neurons between 1 and 4 weeks of cell birth.

A link between neurogenesis and the regulation of granule cell number has recently been proposed and must also be considered. As the volume of the DG was not determined here, it cannot be ruled out that 5-HT3-OE mice exhibit reduced proliferation as a consequence of a greater number or density of neurons in the DG. A family of endogenous cytokines related to transforming growth factor beta are postulated to send an anti-proliferative signal to help limit division of progenitors when a critical number of cells is reached (60). Conversely, the reduction in proliferation observed for 5-HT3-OE mice may directly influence survival. When proliferation is impaired, survival of newly generated cells is enhanced (61).

The survival results from mice sacrificed at 28 days suggest that the ability of new cells, labeled in the first 7 days, to survive the intervening period of cell death is enhanced for 5-HT3-OE mice relative to

WT regardless of delivery method, or, as discussed previously, some cell death occurs earlier in 5-HT3-OE mice. However, survival rates reported here are consistent with studies showing roughly half of the cells born in the DG die between 1 and 2 weeks after their birth (2-4). We found survival ranged from 37-49% for WT and from 49-57% for 5-HT3-OE mice, injection and pump delivery methods respectively. Whatever the explanation for an apparent increase in survival between 1 and 4 weeks, this is a novel finding. Serotonin and receptors for serotonin have been shown to enhance proliferation, leading to increased neurogenesis independent of an effect on survival or differentiation (21, 31, 32). The 5-HT3 receptor may be an important factor mediating survival and differentiation.

The improvement in hippocampal-hippocampal dependent learning noted in 5-HT3-OE mice may provide an explanation for enhanced survival of newly generated cells between 1 and 4 weeks. 5-HT3-OE mice were shown to have improved hippocampal-dependent fear conditioning, specifically related to acquisition (39), which is proposed to depend on DG neurogenesis (5, 56-58). On the other hand, the behavioral phenotype of 5-HT3-OE mice may directly affect neurogenesis, as increased survival has been reliably found as a result of learning (59). Therefore, changes in neurogenesis related to enhanced survival could result in a concomitant improvement in learning like that observed for 5-HT3-OE mice. Alternately, the behavioral phenotype of improved hippocampal-dependent learning reported for 5-HT3-OE mice may result in enhanced survival and neurogenesis.

It remains to be determined if the effect of injection on the number of labeled cells is due to timing, route of administration, BrdU dose, or a host of other things such as inflammation or response to repeated daily handling. Pump implanted animals were administered lower doses of BrdU SQ in a continuous manner while injected animals received higher doses of BrdU IP in a pulsed manner. BrdU is cleared from the brain within 2 hours of injection (42). The fact that the total number of labeled cells was increased using pump infusion indicates that continuous infusion, even at lower doses, may more clearly label dividing cells as BrdU is available during the entire course of the cell cycle. Differences in the kinetics of cell division could have contributed to differences in labeled cell numbers, but it is difficult to predict how an alteration in the length of either S or G0 might differentially affect the labeling of newly generated cells in 5-HT3-OE and WT animals. A toxic effect of high doses of BrdU cannot be discounted as a contributing factor to reduced labeling when BrdU is injected IP, however the doses used in these studies have not been shown to be toxic in rodents (42). SQ delivery avoids first pass metabolism by the liver, whereas IP does not, therefore the percentage of BrdU reaching the brain may be greater when pump infusion is used. Finally, the stress response of the animal to repeated handling and injection may reduce neurogenesis due to glucocorticoid production (62). A recent study by Pencea et al. (63) indicates that intracerebroventricular (ICV) infusion of BrdU via mini-osmotic pump better labels the parenchyma of the brain when compared to IP injection. We report here that ICV infusion is not necessary and SQ infusion labels dividing cells in the DG.

Differences in differentiation were found for genotype, as well as BrdU delivery method. Previous reports indicate that approximately 70% of surviving cells display neuronal markers, 10-15% display

astroglial markers and the remaining do not express markers for neurons or astrocytes and are of an undetermined phenotype (3, 5, 8, 42, 51, 52). 5-HT3-OE mice show a significant reduction in new cells of undetermined phenotype and a concomitant, though slight, increase in new neurons (BrdU/NeuN+). Results indicate that 5-HT3-OE mice have a tendency towards increased neurogenesis mediated by enhanced survival and neuronal differentiation in the face of reduced proliferation – in effect, enhanced neurogenic efficacy. The effect of repeated injection on differentiation was noted for both 5-HT3-OE and WT when compared to the use of an osmotic pump. Injection significantly reduced the number of new neurons detected and increased the number of new cells of undetermined phenotype. The number of new GFAP+ astroglia, though lower than the percent often reported, was not influenced by genotype or delivery method.

Differentiation was affected by BrdU delivery method. When delivering BrdU via IP injection, BrdU+ cells of undetermined phenotype were significantly increased and BrdU+ new neurons significantly decreased. The reason for this is not immediately clear. Interestingly, repeated IP injections introduce LPS into the peritoneum, as LPS is ubiquitous and present in distilled de-ionized water used for preparation of the solution. This may lead to activation of the immune response, resulting in an increase in cytokines, which could then impact the brain, particularly by altering serotonin levels (64). While the permeability of the blood-brain barrier (BBB) may or may not be disrupted by small doses of LPS injected IP (65, 66), the vagus nerve has been shown to convey an inflammatory response to the brain (67). Inflammation in the adult brain activates microglia, which directly, drastically and specifically impairs the development of new neurons, irrespective of tissue damage, likely by the production of cytokines such as tumor necrosis factor, Il-1B and IL-6 which impair neurogenesis (68, 69). IL-6 may inhibit new neuron production by diverting the differentiation of stem cells into glial lineages (68, 70, 71). The drastic nature of reduction of new neurons in response to inflammation suggests that even small amounts of cytokines released from activated microglia could impact neurogenesis. The effect of repeated injections of PBS and LPS-free water has been compared looking at cytokines in whole brain supernatant from 5-HT3-OE and WT mice and preliminary results suggest WT mice have greater production of cytokines in brain following repeated injection (our unpublished observations).

Psychiatric illnesses are accompanied by abnormal signs with counterparts in other species referred to as endophenotypes (72). Immobility in the FST is purported to be an endophenotye reflecting an outward motivational state of behavioral despair (24, 44, 73-76). LH, similarly, has striking face validity and pharmacological specificity as an animal model of depression (77, 78). Results presented here indicate that over-expression of the 5-HT3 receptor leads to antidepressant-like behavior in these tests. Immobility time in these tests may be affected by locomotor changes (73), however there is no change in 5-HT3-OE locomotor activity in an open field (39, 79, 80) when compared to WT, indicating that locomotor activity did not contribute to the immobility scores in FST or escape behavior in the LH active avoidance test. A previous study in rat has assessed the antidepressant effect of an agonist to

the 5-HT3 receptor and demonstrated a dose-dependent antidepressant-like effect of the agonist in the FST (40). 5-HT3 receptor function is potentiated in the CA1 region of the hippocampus following electroconvulsive shock used for drug resistant depression (81), indicating that activation of non-transgenic, endogenous receptor may mediate antidepressant effects. With the use of two depression paradigms, confidence that the phenotype might be relevant to stress-evoked depression can be enhanced (72). Results from the current studies indicate that over-expression of the 5-HT3 receptor produces an antidepressant effect which may be related to increased survival of newly generated cells in the adult DG.

Enhanced survival of newly generated cells may be related to antidepressant-like behavior. Antidepressant treatment increases neurogenesis (20). Decreased proliferation caused by inescapable footshock and the associated helpless behavior are reversed by the SSRI fluoxetine (82). However, helpless behavior appears before proliferation decreases, and rats which don't develop LH do not have reduced proliferation when compared to those that develop LH (83). As changes in differentiation or survival have not been carefully assessed in relation to depression paradigms, it is possible that antidepressant-like behavior may be correlated with neurogenic efficacy or the neurogenic vigor of new born cells, reflected by the percentage of survival. These studies suggest that reduced proliferation, by itself, may not be the best indicator of depression-like behavior. Rather, survival and differentiation effects may also influence behavior in depression paradigms.

Inescapable stress, like that used in LH, reduces proliferation over time (82), and increases dopamine (DA) and serotonin acutely (84). As serotonin is known to enhance proliferation and neurogenesis, the effects of DA on neurogenesis may be important in relation to decreased proliferation. Various drugs of abuse, proposed to exert their effects through the release of dopamine, have been shown to decrease proliferation. Chronic administration of opiates and ethanol decrease neurogenesis (85, 86). The 5-HT3 receptor is thought to play a role in the reward pathway and drug abuse by modulating dopamine release, and brain slices from 5-HT3-OE mice release more DA in response to agonist and cocaine (79). This suggests that DA may mediate the effects of 5-HT3 receptor over-expression on proliferation. The effects of DA on adult neurogenesis have not been adequately explored. However, DA is known to modulate the cell cycle in the embryonic neostriatum. D1-like receptor activation reduces entry of progenitor cells from the G0 to the S-phase (Ohtani et al 2003). It is possible that DA release underlies the reduction in proliferation noted for 5-HT3-OE mice.

In summary, over-expression of the 5-HT3 receptor results in reduced proliferation of cells in the adult DG and enhanced survival such that the final number of new neurons produced does not significantly differ from WT littermates. Furthermore, the enhanced efficiency of neurogenesis correlates with a depression-resistant phenotype in the FST and LH models of depression. These data suggest that changes in adult DG proliferation cannot be used to predict antidepressant-like phenotypes. Rather, neurogenic efficacy may more closely predict behavior in depression paradigms.

Key Research Accomplishments

- Breed the transgene on to the background strains out for 5 generations.
- Test the two bottle choice drinking on the wild type and transgenic N3 and N5 generations of the two strains.
- Test learning performance behavior on the wild type and transgenic N1-N3 N5 strains.
- Identified an effect of the 5HT3 receptor on anxiety related behavior.
- Identify key effect of 5HT3 receptor on inspective behavior
- Characterize unique learning and stress responding in the mice which impacts consumption and aggression.
- Completed alcohol induced aggressive behavior testing on the N1 strains.
- Completed work on neurogenesis and depression and the role of 5HT3 receptors

Reportable Outcomes

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Choi, I.Y., **Allan, A.M**., Zhao, X., Cunningham, L.A. Fetal Alcohol Impairment of Adult Neurogenesis in Mice. Society for Neuroscience Annual Meeting, San Diego, CA 2004

Choi, I.Y., **Allan, A.M**., and Cunningham, L.A. Moderate Fetal Alcohol Exposure (FAE) Impairs the Neurogenic Effect of an Enriched Environment in Mice. Federation of European Neuroscience Society Meeting, Lisbon, Portugal 2004

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